25. A method of labeling an expressed protein, comprising:

(a) expressing a protein linked to a thiol inducible cleavage agent; and cleaving the protein in the presence of a thiol reagent so as to form an expressed protein with a C-terminal thioester

(b) preparing a synthetic peptide fragment having a marker and an N-terminal cysteine; and

(c) ligating the protein with the synthetic peptide to label the protein.

26. The method according to claim 24, wherein the marker is selected from a fluorescent marker, a spin label, an affinity tag, and a radiolabel.

27. The method according to claim 24, wherein the peptide fragment is an antigenic determinant.

A marked-up version of the claims is attached hereto.

REMARKS

Applicants thank the Examiner for the courtesy of an interview on June 20, 2002 when the present claims were discussed. The Examiner indicated that he could allow claim 7 prior to amendment. This claim therefore has been amended to incorporate the limitations of claim 1.

Claims 1-6 and 8-11 have been canceled and new claims 12-27 have been added. Support for the claims include the following:

For claim 12, see page 11, for claim 13, see page 7, for claim 14, see page 7, for claims 15 and 16, see pages 15 through 18, for claim 17, see Example VI, for claims 18-21, see Example 1, for claims 22-24, see Example VI, for claim 25-27, see page 8.

CONCLUSION

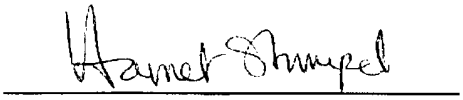
For the reasons set forth above, Applicants respectfully submit that this case is in condition for allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned Attorney would appreciate the opportunity to do so. Thus, the Examiner is hereby authorized to call the undersigned collect at the number shown below.

Respectfully submitted,

NEW ENGLAND BIOLABS, INC.

Date: June 27, 2002



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MARKED-UP VERSION OF THE CLAIMS

7. (amended) A cyclic protein produced by [the method of claim 1] fusing an expressed protein with a peptide, using a method comprising:

(a) generating at least one C-terminal thioester tagged protein;

(b) generating at least one target peptide having a specified N-terminal; and

(c) ligating the target peptide to the target protein.

12. (new) A method for obtaining an expressed protein with a C-terminal thioester, comprising:

(a) obtaining the expressed precursor protein, the precursor having an intein, an intein derivative or mutants thereof; and

(b) reacting the precursor protein with a thiol reagent so as (i) to remove the intein and (ii) to obtain the expressed protein with the C-terminal thioester.

13. (new) The method according to claim 12, wherein the intein is selected from Sce Vma and Mxe Gyr A.

14. (new) The method of claim 12, wherein the thiol reagent is selected from 2-mercaptoethanosulfonic acid, thiophenol, dithiothreitol, and 3- mercaptopropionic acid.

15. (new) The method according to claim 12, wherein the precursor protein is selected from a Bst DNA polymerase I large fragment, Thioredoxin and a cytotoxic protein.

16. (new) The method according to claim 12, wherein the precursor protein is selected from a maltose binding protein and paramyosin.

17. (new) A method for expressing a protein precursor, comprising:

preparing a plasmid having a multiple cloning site between two restriction endonuclease recognition sites; and

inserting a protein encoding nucleic acid sequence into the plasmid upstream of an intein encoding nucleic acid sequence, wherein the cleavage agent sequence is optionally upstream to a binding protein encoding nucleic acid sequence.

18. (new) The method of claim 17, wherein the binding protein encoding nucleic acid sequence is chitin binding protein encoding nucleic acid sequence.

19. (new) The method according to claim 17, wherein the multiple cloning site contains a linker sequence.

20. (new) The method according to claim 19, wherein the linker sequence is selected from SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.

21. (new) The method according to claim 17, wherein the plasmid is a pTXB plasmid.

22. (new) A method of ligating a synthetic fragment in vitro to an inactive expressed protein so as to restore protein activity, comprising:

(a) expressing an inactive truncated form of the protein linked to a thiol inducible cleavage agent; and cleaving the protein in the presence of a thiol reagent so as to form an expressed protein with a C-terminal thioester

(b) preparing a synthetic peptide having an N-terminal cysteine; and

(c) ligating the inactive form of the protein with the synthetic peptide to restore protein activity.

23. (new) The method of claim 20, wherein the protein is a cytotoxic protein.

24. (new) The method of claim 21, wherein the cytotoxic protein is a restriction endonuclease.

25. (new) A method of labeling an expressed protein, comprising:

(a) expressing a protein linked to a thiol inducible cleavage agent; and cleaving the protein in the presence of a thiol reagent so as to form an expressed protein with a C-terminal thioester;

(b) preparing a synthetic peptide fragment having a marker and an N-terminal cysteine; and

(c) ligating the protein with the synthetic peptide to label the protein.

26. (new) The method according to claim 24, wherein the marker is selected from a fluorescent marker, a spin label, an affinity tag, and a radiolabel.

27. (new) The method according to claim 24, wherein the peptide fragment is an antigenic determinant